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# Operational Performance and Turnaround Time of a Tuberculosis Diagnosis Laboratory in Panama

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## Abstract

**Background:** Tuberculosis (TB) diagnosis relies on the coordinated use of rapid molecular testing, microscopy, culture, and drug-resistance assays. This study evaluated laboratory performance and turnaround time (TAT) across an integrated TB diagnostic workflow in Colón Province, Panama, and compared Ziehl–Neelsen (ZN) and auramine–rhodamine microscopy. **Methods:** A retrospective laboratory-based study included 501 unique patients with positive Xpert MTB/RIF Ultra results between January 2021 and December 2025. The routine workflow included ZN microscopy, Xpert MTB/RIF Ultra, BD BACTEC MGIT 320 culture, and post-culture GenoType MTBDRplus testing. A separate microscopy analysis included 300 specimens with sufficient residual material for paired ZN and auramine–rhodamine staining, using Xpert MTB/RIF Ultra as the reference comparator. **Results:** ZN microscopy was positive in 96.4% (483/501) of Xpert-positive specimens, and ZN positivity increased significantly across Xpert semiquantitative categories ($p$ for trend < 0.001). Among specimens with final positive MGIT cultures, increasing Xpert semiquantitative category was strongly associated with shorter MGIT TAT (Spearman's $\rho = -0.728$, $p < 0.001$). Initial MGIT contamination occurred in 5.2% (25/480) of cultures; cultures recovered after contamination had a longer median TAT than those without initial contamination (24 vs. 16 days, $p < 0.001$). Auramine–rhodamine microscopy showed higher sensitivity than ZN microscopy [96.8% (95% CI, 92.8–98.6) vs. 88.0% (95% CI, 82.0–92.2)] and stronger agreement with Xpert [$\kappa = 0.967$ (95% CI, 0.938–0.996) vs. 0.874 (95% CI, 0.819–0.928)]. **Conclusions:** The integrated laboratory workflow supported rapid initial TB diagnosis while maintaining culture and drug-resistance testing capacity. Molecular bacterial burden was associated with smear positivity and culture TAT, while fluorescence microscopy showed improved performance relative to ZN microscopy. Culture recovery procedures preserved diagnostic yield despite delays related to contamination.

**Keywords:** tuberculosis; laboratory performance; turnaround time; Xpert MTB/RIF Ultra; MGIT; GenoType MTBDRplus; auramine–rhodamine; Ziehl–Neelsen; drug resistance; Panama

Academic Editors: Christoph Seger and Jong Seok Lee

Received: 4 September 2026
Revised: 23 October 2026
Accepted: 26 October 2026
Published: 18 November 2026

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## 1. Introduction

Tuberculosis (TB) remains a major global public health challenge and one of the leading causes of death from infectious disease [1]. Timely bacteriological confirmation and early detection of drug resistance are essential for effective treatment and transmission control [2]. Current World Health Organization (WHO) recommendations prioritize rapid molecular assays for the initial detection of *Mycobacterium tuberculosis (M. tuberculosis)* and rifampicin resistance, while smear microscopy, liquid culture, and molecular drug-resistance testing continue to provide complementary information within the diagnostic pathway [3]. Consequently, laboratory performance depends not only on the analytical characteristics of individual tests but also on how efficiently these methods are integrated and reported [4].

Turnaround time (TAT) is an operational measure of the time elapsed between a predefined starting point in the laboratory pathway and the release of the corresponding diagnostic results. In TB diagnostics, the relevant starting point may differ by diagnostic stage; therefore, TAT should be defined separately for rapid testing, culture, and post-culture drug-resistance testing [5]. Rapid molecular assays and smear microscopy can provide results early in the diagnostic pathway, whereas liquid culture and culture-dependent resistance testing require substantially longer processing intervals [3,4]. Consequently, specimen availability, culture contamination, laboratory workflow, and access to downstream drug-susceptibility testing can all influence the overall time required to obtain complete microbiological information. Although molecular assays are prioritized for initial diagnosis, smear microscopy remains widely used in routine TB laboratories because it is rapid, simple, and requires relatively few resources. Fluorescence-based staining methods, such as auramine–rhodamine, may improve bacillary detection and enable more efficient slide screening than conventional ZN microscopy [6,7]. Liquid culture is also vulnerable to contamination, which can delay or prevent a reportable result unless appropriate recovery procedures are available [8].

Evaluating the complete diagnostic workflow may therefore provide a more informative assessment of laboratory performance than evaluating individual methods in isolation. This is particularly relevant for regional laboratories that integrate rapid diagnosis with culture, resistance testing, and referral to national reference laboratories.

In the Americas, TB continues to affect vulnerable populations disproportionately, and important gaps in diagnosis persist [9]. Panama continues to report a TB incidence of approximately 45 cases per 100,000 population, while Colón Province has historically carried a substantial disease burden [10,11]. Molecular epidemiological studies in Colón have documented sustained circulation of *M. tuberculosis* Lineage 2 strains, with recent genomic surveillance identifying endemic transmission of the L2.2.M3 sublineage [12,13]. Lineage 2 has been associated with rapid transmission and drug resistance, reinforcing the importance of timely diagnosis and resistance detection in this setting [13]. The Laboratorio Interinstitucional Regional de Tuberculosis (LIRT) integrates ZN microscopy, Xpert MTB/RIF Ultra, liquid culture, and molecular drug-resistance testing within a regional laboratory network. However, operational evidence describing the performance and TAT of this integrated pathway remains limited. This study therefore aimed to evaluate the operational performance and TAT of the TB diagnostic workflow at LIRT among patients with Xpert MTB/RIF Ultra-positive results. A secondary analysis compared the diagnostic performance of ZN and auramine-rhodamine microscopy using Xpert MTB/RIF Ultra as the reference method. Together, these analyses provide an integrated assessment of laboratory workflow, TAT, and microscopy performance within a regional TB diagnostic setting.

## 2. Materials and Methods

### *2.1. Study Design and Laboratory Settings*

This retrospective, laboratory-based study was conducted at the LIRT in Colón Province, Panama, from January 2021 to December 2025. LIRT operates through a collaborative partnership among the Ministerio de Salud (MINSA), Caja de Seguro Social (CSS), and Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT-AIP), providing routine TB diagnostic services through smear microscopy, molecular testing, liquid culture, and molecular drug-resistance testing. LIRT receives specimens from the main referral hospital, primary healthcare facilities, and other healthcare services within Colón Province. During the study period, the LIRT registered approximately 5672 pulmonary and extrapulmonary specimens for TB investigation. All routine diagnostic procedures included in the primary operational analysis were performed within the LIRT laboratory workflow. Referral to the National Reference Laboratory for Tuberculosis at the Laboratorio Central de Referencia en Salud Pública of the Instituto Conmemorativo Gorgas de Estudios de la Salud (LCRSP-ICGES) occurred only when additional second-line drug susceptibility testing was required; therefore, the primary workflow evaluated in this study represents a single regional laboratory pathway that independent testing performed across multiple laboratories.

Trained personnel performed laboratory procedures using biosafety level 2 infrastructure and TB-specific containment practices, including Class II biological safety cabinets and procedures designed to minimize aerosol exposure during specimen and culture handling. LIRT participated in external quality assessment (EQA) coordinated by the LCRSP-ICGES. During the study period, available EQA reports evaluated Xpert MTB/RIF/Ultra, GenoType MTBDRplus, and mycobacterial culture using standardized reference panels, predefined performance criteria, and documented results in standardized reports.

### *2.2. Study Population and Analytical Components*

The study comprised two analytical components. The primary analysis evaluated the operational laboratory workflow and turnaround time among 501 unique patients with positive Xpert MTB/RIF Ultra results identified between January 2021 and December 2025.

Accordingly, the primary operational cohort represents a selected population of bacteriologically confirmed TB cases and does not represent the full spectrum of specimens submitted to LIRT for TB investigation during the study period.

This primary operational cohort included only Xpert MTB/RIF Ultra-positive patients and therefore evaluated laboratory workflow, turnaround time, culture outcomes, and downstream drug-resistance testing rather than diagnostic accuracy. We evaluated sensitivity, specificity, and other diagnostic performance measures only in a separate comparative microscopy dataset that included both Xpert-positive and Xpert-negative specimens.

Each patient contributed a single clinical specimen to the primary operational cohort; therefore, no patient was represented by more than one specimen in the analysis. We performed MGIT culture when sufficient residual specimen volume remained after the initial diagnostic procedures. Consequently, we could culture 480 of the 501 patients in the primary operational cohort; in 21 cases, we could not perform MGIT culture because of insufficient specimen volume.

Separately, we conducted a comparative microscopy analysis using 300 clinical specimens with sufficient residual material for both Ziehl–Neelsen and auramine–rhodamine staining. We selected specimens based solely on the availability of sufficient residual material for both staining procedures and did not use random sampling. The microscopy

dataset included both Xpert MTB/RIF Ultra-positive and Xpert MTB/RIF Ultra-negative specimens and was analyzed independently from the primary operational cohort. Microscopy readings were performed blinded to Xpert MTB/RIF Ultra results.

Inclusion and Exclusion Criteria

For the primary operational cohort, patients were eligible if they had a positive Xpert MTB/RIF Ultra result at LIRT between January 2021 and December 2025. Each patient contributed one clinical specimen, and no patient contributed more than one specimen to the analysis. We excluded Xpert MTB/RIF Ultra-negative specimens from the primary operational cohort because this component evaluated downstream laboratory workflow among bacteriologically confirmed cases rather than diagnostic accuracy. For the comparative microscopy analysis, specimens were eligible when sufficient residual material was available to perform both ZN and auramine–rhodamine staining and when an Xpert MTB/RIF Ultra result was available for comparison. Specimens with insufficient residual material to perform both staining procedures were not included in the comparative microscopy dataset. Selection was based on residual specimen availability and was not random.

### *2.3. Laboratory Diagnostic Workflow and Turnaround Time*

The routine laboratory workflow at LIRT included Ziehl–Neelsen (ZN) smear microscopy, Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA), liquid culture using the BD BACTEC™ MGIT™ 320 system (MGIT), and post-culture molecular drug-resistance testing using GenoType MTBDRplus v2.0. Before 2021, LIRT did not have GenoType MTBDRplus, and specimens requiring molecular drug-resistance testing were referred to the LCRSP-ICGES. After local implementation, LIRT incorporated GenoType MTBDRplus into its routine diagnostic workflow. LIRT referred isolates with detected resistance requiring further evaluation of susceptibility to second-line anti-tuberculosis drugs to LCRSP-ICGES, as LIRT did not have second-line drug-susceptibility testing available. When primary MGIT cultures were contaminated and sufficient residual specimen was available, laboratory staff reprocessed and reinoculated the specimen into a new MGIT tube according to routine laboratory procedures.

We defined turnaround time (TAT) separately for each diagnostic stage, based on its operational start and end points. For ZN smear microscopy and Xpert MTB/RIF Ultra, TAT was defined as the interval from specimen receipt at LIRT to release of the corresponding laboratory result and was categorized as <8 h, 8–16 h, 16–24 h, and >24 h. We first prepared ZN smears, followed immediately by Xpert MTB/RIF Ultra testing within the same specimen-processing episode; consequently, both methods generally fell into the same operational TAT category. For MGIT culture, TAT was defined as the interval from specimen receipt at LIRT to reporting of the final culture result and was expressed in days. For GenoType MTBDRplus, post-culture TAT was defined as the interval from MGIT culture positivity to release of the GenoType MTBDRplus result. Because MGIT and GenoType MTBDRplus TAT represent different stages of the diagnostic workflow and start at different points, we analyzed these intervals separately and did not compare them statistically.

More granular timestamps, including specimen receipt-to-registration, registration-to-processing, assay completion-to-result validation, and result release-to-clinician notification, were not consistently recorded in the retrospective laboratory dataset and therefore could not be reconstructed reliably. Consequently, we evaluated TAT at the predefined diagnostic-stage level rather than decomposing it into individual intra-laboratory processing intervals.

We evaluated available laboratory quality indicators by diagnostic pathway stage. Pre-analytical indicators included insufficient residual specimen volume preventing MGIT culture. Analytical indicators included initial MGIT contamination and successful culture recovery after reprocessing and reinoculation. Post-analytical indicators included reporting turnaround time for rapid diagnostics, culture, and post-culture molecular resistance testing. These indicators were calculated within the corresponding analytical cohorts and should not be interpreted as laboratory-wide rates for all specimens submitted to LIRT during the study period.

### *2.4. Comparative Microscopy Analysis*

For comparative microscopy analysis, we performed Ziehl–Neelsen and auramine–rhodamine staining on the same specimen whenever sufficient residual material was available. Microscopy readings were interpreted independently and blinded to Xpert MTB/RIF Ultra results. We selected Xpert MTB/RIF Ultra as the reference comparator because it was the routine molecular diagnostic test available for all specimens in the comparative microscopy dataset. It was not considered an absolute microbiological gold standard; therefore, this analysis reports diagnostic performance relative to Xpert MTB/RIF Ultra.

Diagnostic performance measures included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy, and Cohen's kappa coefficient. We evaluated paired categorical results using McNemar's test and assessed differences in bacillary grading between staining methods using the Wilcoxon signed-rank test.

### *2.5. Statistical Analysis*

Categorical variables were summarized as frequencies and percentages. Continuous turnaround-time variables were summarized using medians, interquartile ranges (IQRs), and ranges. We compared MGIT turnaround times between cultures without initial contamination and cultures successfully recovered after initial contamination using the Wilcoxon rank-sum test. We also performed exploratory analyses of MGIT contamination. We compared contamination frequencies by pulmonary versus extrapulmonary specimen classification, sputum versus non-sputum specimen type, and HIV status using Fisher's exact test. Temporal trends in contamination across calendar years were evaluated using the Cochran–Armitage trend test. Potential seasonality was explored by comparing contamination frequencies across calendar quarters using Fisher's exact test.

We further explored associations between Xpert MTB/RIF Ultra semiquantitative categories and bacteriological indicators. Trends in ZN smear positivity across ordered Xpert semiquantitative categories were evaluated using the Cochran–Armitage trend test. Among specimens with a final positive MGIT culture, the association between Xpert semiquantitative category and MGIT TAT was assessed using Spearman's rank correlation coefficient.

For the comparative microscopy analysis, we calculated sensitivity, specificity, PPV, NPV, overall accuracy, and Cohen's kappa for ZN and auramine–rhodamine staining, using Xpert MTB/RIF Ultra as the reference method. We calculated two-sided 95% confidence intervals (95% CIs) for sensitivity, specificity, PPV, NPV, and overall accuracy using the Wilson score method, and 95% CIs for Cohen's kappa using the asymptotic standard error. We compared paired categorical results using McNemar's test, and we assessed differences in bacillary grading using the Wilcoxon signed-rank test. We considered a two-sided $p$-value $< 0.05$ statistically significant. All analyses were performed using R software, version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

### *3.1. Study Population and Specimen Characteristics*

We included 501 unique patients with a positive Xpert MTB/RIF Ultra result in the analysis. Age information was available for 496 patients, with a median age of 45 years (IQR, 32–59; range, 3–90 years). Most patients were male 347 (69.3%), and 316 (63.1%) were hospitalized at the time of specimen collection, whereas 185 (36.9%) were managed in outpatient primary care settings.

Most specimens were pulmonary 482 (96.2%) with sputum representing the predominant specimen type 453 (90.4%), followed by bronchial lavage 26 (5.2%). Extrapulmonary specimens accounted for 19 (3.8%) of the study samples. HIV infection was documented in 25 patients (5.0%), while reported drug use was present in 9 (1.8%). Table 1 shows patient information.

**Table 1.** Demographic and specimen characteristics of the primary operational cohort (n = 501), 2021–2025.

| Characteristic | Value |
|---|---|
| Age (years), median (IQR; min–max) | 45 (32–59; 3–90) |
| Male sex, *n* (%) | 347 (69.3) |
| Female sex, *n* (%) | 154 (30.7) |
| HIV-positive, *n* (%) | 25 (5.0) |
| Drug use, *n* (%) | 9 (1.8) |
| Hospitalized, *n* (%) | 316 (63.1) |
| Outpatient/primary care, *n* (%) | 185 (36.9) |
| Pulmonary specimen, *n* (%) | 482 (96.2) |
| Extrapulmonary specimen, *n* (%) | 19 (3.8) |
| Sputum, *n* (%) | 453 (90.4) |
| Bronchial lavage, *n* (%) | 26 (5.2) |

IQR, interquartile range; HIV, human immunodeficiency virus.

### *3.2. Laboratory Findings and Culture Outcomes*

ZN smear microscopy detected acid-fast bacilli in 483 of 501 Xpert-positive specimens (96.4%), whereas 18 (3.6%) were smear-negative. Xpert MTB/RIF Ultra semiquantitative results were classified as trace in 10 (2.0%), very low in 41 (8.2%), low in 143 (28.5%), medium in 116 (23.2%), and high in 191 (38.1%) patients. Overall, 307 of 501 patients (61.3%) had medium or high semiquantitative Xpert results.

Xpert MTB/RIF Ultra detected rifampicin resistance in 25 patients (5.0%), did not detect it in 466 (93.0%), and reported indeterminate results in 10 (2.0%).

MGIT culture was performed in 480 of the 501 specimens; culture could not be performed in 21 cases (4.2%) because of insufficient specimen volume. Of the cultures performed, 445 yielded growth without initial contamination, 25 (5.2%) were initially contaminated, and 10 showed no growth. Of the 25 initially contaminated cultures, 23 (92.0%) were successfully recovered after reprocessing and reinoculation and subsequently grew, whereas two showed no growth on repeat culture. Overall, 468 of 480 cultures performed (97.5%) ultimately yielded a positive MGIT result, while 12 (2.5%) showed no growth. Among cultures that ultimately yielded growth, the median time to MGIT result was 17 days (IQR, 12–23; range, 3–29 days). Cultures requiring recovery after an initial contamination event had a longer median TAT of 24 days (IQR, 23–26), compared with 16 days (IQR, 12–22) among cultures without contamination (Wilcoxon rank-sum test, $p < 0.001$).

Exploratory analysis showed no evidence that initial MGIT contamination differed between pulmonary and extrapulmonary specimens [24/463 (5.2%) vs. 1/17 (5.9%),

respectively; Fisher's exact $p = 0.604$], or between sputum and non-sputum specimens [23/437 (5.3%) vs. 2/43 (4.7%); $p = 1.000$]. Contamination was also similar among HIV-positive and HIV-negative patients [1/24 (4.2%) vs. 24/455 (5.3%); $p = 1.000$]. Among cultures with an available specimen-receipt date, contamination decreased from 12.3% (7/57) in 2021 to 1.4% (1/74) in 2025, with a significant decreasing trend across calendar years (Cochran–Armitage trend test, $p = 0.006$). No significant seasonal variation was observed across calendar quarters ($p = 0.739$).

Genotype MTBDRplus testing was completed for all 468 specimens with a final positive MGIT culture. We detected no resistance-associated pattern in 453 isolates (96.8%), whereas we detected resistance-associated patterns in 13 (2.8%). Two additional isolates (0.4%) lacked the rpoB wild-type band and had no corresponding mutation band. We subsequently referred isolates with detected resistance to LCRSP-ICGES for second-line drug-susceptibility testing. Among the 13 isolates with detected resistance-associated patterns, four showed rpoB MUT2B, one rpoB MUT2B/inhA3, two rpoB MUT3, four rpoB MUT3/katG MUT1, and two rpoB MUT3/katG MUT2. Figure 1 summarizes these laboratory outcomes.

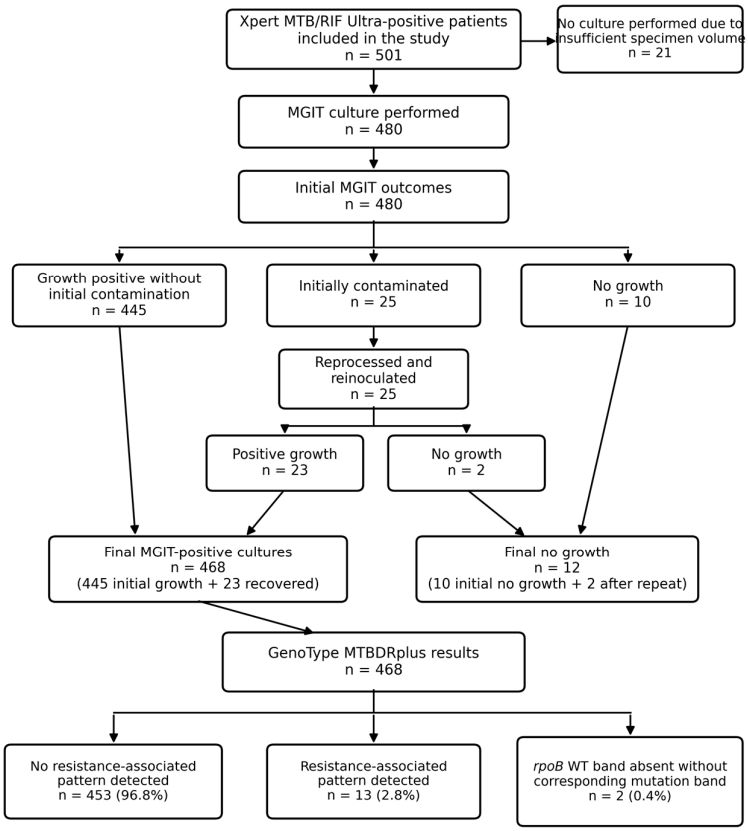



**Figure 1.** Flow of the primary operational cohort through MGIT culture and post-culture GenoType MTBDRplus testing. Of 501 Xpert MTB/RIF Ultra-positive patients, 480 had sufficient specimen volume for MGIT culture, and 21 did not. Of the 480 cultures, 445 grew without initial contamination, 25 were initially contaminated, and 10 showed no growth. After reprocessing and reinoculating contaminated cultures, 23 showed positive growth, and two showed no growth, resulting in 468 final MGIT-positive cultures and 12 cultures with no growth. All 468 MGIT-positive cultures underwent GenoType MTBDRplus testing.

### *3.3. TAT Across the Diagnostic Workflow*

Rapid diagnostic testing showed short reporting times: 362 of 501 results (72.3%) were reported within 8 h, 36 (7.2%) within 8–16 h, 15 (3.0%) within 16–24 h, and 88 (17.6%) after 24 h. The same categorical TAT distribution was observed for ZN smear microscopy and Xpert MTB/RIF Ultra, reflecting their parallel processing within the routine laboratory workflow as shown in Table 2.

**Table 2.** Available laboratory quality indicators within the study cohorts.

| Quality stage | Indicator | Result |
|---|---|---|
| Pre-analytical | Insufficient residual volume preventing MGIT culture | 21/501 (4.2%) |
| Analytical | Initial MGIT contamination | 25/480 (5.2%) |
| Analytical | Successful recovery after initial contamination | 23/25 (92.0%) |
| Post-analytical | ZN/Xpert results reported in <8 h | 362/501 (72.3%) |
| Post-analytical | Median MGIT TAT | 17 days |
| Post-analytical | Median post-culture GenoType MTBDRplus TAT | 27 days |

Indicators are reported for the corresponding study cohorts and do not represent laboratory-wide quality rates for all specimens processed at LIRT. MGIT, Mycobacteria Growth Indicator Tube; TAT, turnaround time; ZN, Ziehl–Neelsen.

Among the 468 specimens with a positive final MGIT culture, the median time from specimen receipt to the MGIT result was 17 days (IQR, 12–23; range, 3–29 days). Post-culture GenoType MTBDRplus TAT, defined as the interval from MGIT culture positivity to release of the GenoType MTBDRplus result, had a median of 27 days (IQR, 19–32; range, 10–53 days). This interval does not represent time from specimen receipt or culture inoculation; it specifically reflects the post-culture molecular testing stage.

Because these intervals represented different stages of the diagnostic workflow, we did not directly compare MGIT and GenoType TAT as shown in Table 3.

**Table 3.** Rapid diagnostic testing TAT. ZN smear microscopy and Xpert MTB/RIF Ultra were processed in parallel as part of the routine laboratory workflow, resulting in the same categorical TAT distribution.

| Method | <8 h, n (%) | 8–16 h, n (%) | 16–24 h, n (%) | >24 h, n (%) |
|---|---|---|---|---|
| **ZN smear microscopy (n = 501)** | 362 (72.3) | 36 (7.2) | 15 (3.0) | 88 (17.6) |
| **Xpert MTB/RIF Ultra (n = 501)** | 362 (72.3) | 36 (7.2) | 15 (3.0) | 88 (17.6) |

**TAT**, turnaround time; **IQR**, interquartile range; **ZN**, Ziehl–Neelsen.

At the stage level, the longest observed intervals occurred during culture and post-culture molecular resistance testing (Table 4).

**Table 4.** Culture and post-culture molecular testing. MGIT TAT was measured from specimen receipt to the reportable culture result.

| Diagnostic stage | n | Median, days | IQR, days | Range, days |
|---|---|---|---|---|
| Final positive MGIT cultures | 468 | 17 | 12–23 | 3–29 |
| MGIT-positive cultures without initial contamination | 445 | 16 | 12–22 | 3–29 |
| MGIT cultures recovered after initial contamination | 23 | 24 | 23–26 | 22–27 |
| GenoType MTBDRplus post-culture TAT | 468 | 27 | 19–32 | 10–53 |

We measured GenoType MTBDRplus post-culture TAT from MGIT positivity to release of the molecular drug-resistance result; therefore, MGIT and GenoType TAT represent different stages of the diagnostic workflow and were not directly compared statistically. **MGIT**, Mycobacteria Growth Indicator Tube.

However, because intra-stage timestamps were not consistently available, we could not further partition these intervals to determine whether delays arose from registration, processing schedules, assay batching, result validation, or reporting.

### *3.4. Laboratory Quality Indicators and External Quality Assessment*

Available external quality assessment reports documented consistently high performance across the evaluated laboratory methods. Molecular EQA panels showed 100% concordance in the available GeneXpert evaluations, while GenoType MTBDRplus assessments documented efficiencies of 97.5–100%. The available mycobacterial culture EQA report showed an overall score of 100%, classified as excellent according to the reference laboratory criteria.

### *3.5. Association Between Xpert Semiquantitative Results and Bacteriological Indicators*

ZN positivity increased progressively across Xpert MTB/RIF Ultra semiquantitative categories. Among specimens with trace Xpert results, 3 of 10 (30.0%) were ZN-positive, compared with 34 of 41 (82.9%) very-low, 139 of 143 (97.2%) low, 116 of 116 (100%) medium, and 191 of 191 (100%) high results. A significant increasing trend in ZN positivity was observed across Xpert semiquantitative categories (Cochran–Armitage trend test, $p < 0.001$) See Table 5.

Among the 468 specimens that ultimately yielded positive MGIT cultures, increasing Xpert semiquantitative category was strongly associated with shorter MGIT turnaround time (Spearman's $\rho = -0.728$, $p < 0.001$). Median MGIT TAT was 25 days among trace specimens, 23 days among very-low and low specimens, 18 days among medium specimens, and 11 days among high specimens. These findings demonstrate an inverse relationship between molecular bacterial burden and time to culture detection. See Table 5.

**Table 5.** ZN positivity showed a significant increasing trend across ordered Xpert MTB/RIF Ultra semiquantitative categories (Cochran–Armitage trend test, $p < 0.001$). Among specimens with a final positive MGIT culture, increasing Xpert semiquantitative category was strongly associated with shorter MGIT TAT (Spearman's $\rho = -0.728$, $p < 0.001$).

| Xpert Category | Total, n | ZN Positive, n (%) | Final MGIT-Positive, n | Median MGIT TAT, Days |
|---|---|---|---|---|
| **Trace** | 10 | 3 (30.0) | 4 | 25 |
| **Very low** | 41 | 34 (82.9) | 26 | 23 |
| **Low** | 143 | 139 (97.2) | 134 | 23 |
| **Medium** | 116 | 116 (100) | 114 | 18 |
| **High** | 191 | 191 (100) | 190 | 11 |

**TAT**, turnaround time; **ZN**, Ziehl–Neelsen; **MGIT**, Mycobacteria Growth Indicator Tube.

### *3.6. Comparative Microscopy Analysis*

The comparative microscopy analysis included 300 specimens, of which 158 were positive and 142 negative by Xpert MTB/RIF Ultra. ZN microscopy identified 139 positive and 161 negative specimens, whereas auramine–rhodamine microscopy identified 153 positive and 147 negative specimens.

Auramine–rhodamine demonstrated higher sensitivity than ZN microscopy: 96.8% (95% CI, 92.8–98.6) vs 88.0% (95% CI, 82.0–92.2). Agreement with Xpert MTB/RIF Ultra was also stronger for auramine–rhodamine ($\kappa = 0.967$; 95% CI, 0.938–0.996) than for ZN microscopy ($\kappa = 0.874$; 95% CI, 0.819–0.928).

McNemar's test showed significant discordance between ZN microscopy and Xpert MTB/RIF Ultra ($p < 0.001$), whereas the difference between auramine–rhodamine and

Xpert MTB/RIF Ultra was not statistically significant ($p$ = 0.074). Bacillary grading also differed significantly between the two staining methods (Wilcoxon signed-rank test, $p$ < 0.001, Table S1). Table 6 presents specificity, PPV, NPV, and overall accuracy with corresponding 95% confidence intervals.

**Table 6.** Comparative performance of microscopy methods using Xpert MTB/RIF Ultra as the reference method.

| Performance Measure | Ziehl–Neelsen | Auramine–Rhodamine |
|---|---|---|
| Sensitivity, % (95% CI) | 88.0 (82.0–92.2) | 96.8 (92.8–98.6) |
| Specificity, % (95% CI) | 100 (97.4–100) | 100 (97.4–100) |
| PPV, % (95% CI) | **100 (97.3–100)** | **100 (97.6–100)** |
| NPV, % (95% CI) | 88.2 ***(82.3–92.3)*** | 96.6 *(92.3–98.5)* |
| Accuracy, % (95% CI) | 93.7 *(90.3–95.9)* | 98.3 *(96.2–99.3)* |
| **Cohen's κ (95% CI)** | 0.874 (0.819–0.928) | 0.967 (0.938–0.996) |
| **McNemar *p*-value** | <0.001 | 0.074 |

**CI**, confidence interval; **PPV,** positive predictive value; **NPV**, negative predictive value.

## 4. Discussion

This study identified several findings relevant to the performance of an integrated regional TB laboratory workflow. Molecular bacterial burden, as reflected by Xpert MTB/RIF Ultra semiquantitative categories, was associated with both smear positivity and MGIT turnaround time. Culture contamination introduced additional delays, although most affected cultures were successfully recovered, preserving access to downstream molecular drug-resistance testing. In a separate microscopy analysis, auramine–rhodamine performed better than conventional Ziehl–Neelsen staining. Together, these findings illustrate how bacterial burden, specimen processing, and diagnostic method influence performance across the laboratory pathway and support the integrated use of rapid molecular testing, microscopy, culture, and drug-resistance testing recommended by WHO [2,3]. Framing the available measures as laboratory quality indicators also highlights that performance assessment extends beyond diagnostic accuracy and includes specimen adequacy, contamination control, recovery procedures, and timely reporting across the diagnostic pathway.

The relationship between Xpert semiquantitative category and bacteriological indicators was particularly evident in the primary cohort. Increasing molecular bacterial burden was associated with greater ZN positivity and shorter MGIT turnaround time, consistent with previous evidence linking Xpert semiquantitative categories with smear grade and culture kinetics [14,15]. Therefore, interpret the high overall ZN positivity in the context of a study population restricted to Xpert MTB/RIF Ultra-positive patients, many of whom had medium or high semiquantitative results. Accordingly, the observed ZN positivity should not be interpreted as smear microscopy sensitivity in an unselected population undergoing TB investigation.

Comparative microscopy analysis further showed that auramine–rhodamine microscopy had higher sensitivity and stronger agreement with Xpert MTB/RIF Ultra than conventional ZN microscopy. This finding is consistent with previous evidence showing improved sensitivity of fluorescence microscopy with little or no loss of specificity [7,16]. Fluorescence microscopy also allows larger smear areas to be examined at lower magnification and may reduce slide-reading time [17]. These operational advantages may be relevant in regional laboratories where microscopy remains part of the diagnostic workflow, although implementation requires appropriate equipment, trained personnel,

quality assurance, and maintenance capacity. Thus, fluorescence microscopy should be considered complementary to, rather than a replacement for, molecular testing.

Culture contamination represented an important operational challenge because it affected not only culture TAT but also subsequent stages of the diagnostic pathway. Reprocessing and reinoculation allowed most initially contaminated cultures to be recovered, consistent with previous evidence supporting active management of contaminated MGIT cultures [18]. However, because GenoType MTBDRplus testing could proceed only after a positive MGIT result, contamination-related delays also postponed downstream molecular resistance characterization and, when required, referral for additional drug-susceptibility testing. These findings emphasize the importance of monitoring both contamination rates and their effect on the availability and timing of final laboratory results.

The exploratory contamination analysis did not identify meaningful differences according to pulmonary versus extrapulmonary origin, sputum versus other specimen types, HIV status, or calendar quarter. However, contamination showed a significant decreasing trend over the study period. This temporal improvement may reflect increasing operational consistency and accumulated laboratory experience, although the retrospective design does not allow the specific causes of this reduction to be determined.

Integrating GenoType MTBDRplus into the regional workflow provided an additional level of resistance characterization after culture confirmation. Line probe assays remain useful for detecting resistance-associated patterns involving *rpoB*, *katG*, and the *inhA* promoter region [19,20], although culture-dependent testing inevitably introduces additional time before complete resistance information becomes available [21]. More broadly, TAT differences across the diagnostic pathway reinforce the importance of evaluating the laboratory cascade as a whole rather than focusing exclusively on the analytical speed of individual assays. Laboratory performance is influenced by specimen processing, culture recovery, post-culture testing, result reporting, and referral pathways [5].

The resistance-associated patterns detected in this cohort are also epidemiologically relevant. The rpoB MUT2B and MUT3 probes correspond to the H526D and S531L substitutions, respectively, both well-established markers of rifampicin resistance, while katG MUT1 corresponds to the S315T1 substitution associated with isoniazid resistance. The coexistence of rpoB mutations with katG- or inhA-associated resistance markers therefore supports a molecular MDR-TB pattern, whereas isolated rpoB mutations indicate rifampicin resistance without concomitant isoniazid resistance. Importantly, these patterns are consistent with previous molecular surveillance in Panama, where H526D and S531L were among the most frequent rpoB mutations, and S315T1 was a common katG resistance-associated mutation. The similarity between the present findings and national resistance patterns supports the epidemiological relevance of continued molecular surveillance of first-line drug-resistance mutations in Panama [22].

The prolonged intervals observed during culture and post-culture resistance testing identify these stages as potential targets for operational review. Still, the available retrospective timestamps did not permit attribution of delays to specific processes such as registration, assay scheduling, batching, validation, or clinician notification. Future prospective workflow evaluations should capture these timestamps separately to distinguish unavoidable analytical time from modifiable operational delay.

This study has several limitations. The primary operational cohort included only patients with positive Xpert MTB/RIF Ultra results and therefore represents a selected population of bacteriologically confirmed TB cases rather than the full spectrum of specimens submitted to LIRT for TB investigation. Consequently, we interpret findings on culture yield, contamination, and TAT within this selected cohort. They cannot be generalized to overall laboratory performance across all specimens processed during this study period.

The available retrospective dataset lacked sufficiently complete stage-specific operational data for all specimens submitted to the laboratory to reconstruct the same workflow indicators across the entire laboratory population. We restricted diagnostic performance estimates to the separate comparative microscopy analysis, which included both Xpert-positive and Xpert-negative specimens. In addition, we selected specimens for microscopy analysis based on residual specimen availability rather than random sampling, which may introduce selection bias and limit the generalizability of these estimates. We used Xpert MTB/RIF Ultra as the reference comparator rather than an absolute microbiological gold standard; therefore, interpret microscopy performance estimates relative to Xpert MTB/RIF Ultra.

## 5. Conclusions

This study showed that an integrated regional TB laboratory workflow can support rapid initial diagnosis while maintaining culture and molecular drug-resistance testing. Higher Xpert MTB/RIF Ultra semiquantitative categories were associated with greater ZN smear positivity and shorter MGIT turnaround time. Culture contamination prolonged the diagnostic pathway, although reprocessing successfully recovered most affected cultures. In the comparative microscopy analysis, auramine–rhodamine showed higher sensitivity and stronger agreement with Xpert MTB/RIF Ultra than conventional ZN microscopy. These findings identify bacterial burden, culture contamination, and microscopy method as factors that influence laboratory performance.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/diagnostics16223868/s1, Table S1. Bacillary-grade distribution by Ziehl–Neelsen and auramine–rhodamine microscopy.

**Author Contributions:** Conceptualization, P.P., M.V., L.S., J.J. and A.G.; methodology, P.P., M.V., L.S., L.G., F.A., J.J. and A.G.; software, P.P.; validation, P.P., M.V., L.S., L.G., G.C., T.L., Y.C., F.A., D.S., J.E.K., A.A.G., O.L., A.d.C., N.T., J.J. and A.G.; formal analysis, P.P., M.V., L.S., J.J. and A.G.; investigation, P.P., M.V., L.S., L.G., G.C., T.L., Y.C., F.A., D.S., J.E.K., A.A.G., O.L., A.d.C., N.T., J.J. and A.G.; resources, P.P., M.V., L.G., G.C., T.L., Y.C., F.A., D.S., J.E.K., A.A.G., O.L., A.d.C., N.T., J.J. and A.G.; data curation, P.P., M.V., L.S., L.G., G.C., T.L., Y.C., F.A., D.S., J.E.K., A.A.G., O.L., A.d.C., N.T., J.J. and A.G.; writing—original draft preparation, P.P.; writing—review and editing, P.P., M.V., L.S., L.G., G.C., T.L., Y.C., F.A., D.S., J.E.K., A.A.G., O.L., A.d.C., N.T., J.J. and A.G.; visualization, P.P.; supervision, P.P., J.J. and A.G.; project administration, P.P., M.V., J.J. and A.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the Sistema Nacional de Investigación (SNI) and FIED grant No. 137-2023. Laboratory infrastructure was supported by the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) through project INF-11-39.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of Universidad Santander, Panama (protocol codes CBIUSantander-M-041-2022, approval date: 18 May 2022; CBI-USantander-M-081-2022, approval date: 13 September 2022; and CBI-USantander-M-108-2025, approval date: 11 August 2025).

**Informed Consent Statement:** Informed consent for participation was obtained from all subjects involved in the study.

**Data Availability Statement:** The data supporting the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available because they contain information derived from routine clinical and laboratory records and are subject to institutional data protection and confidentiality requirements.

https://doi.org/10.3390/diagnostics16223868

**Acknowledgments:** We thank Jaime Bravo, Jaime Miranda, Neila Barios, Lee Riley (deceased), Laura Flores, Ricardo Lleonart, and Jagannatha Rao for their valuable support and guidance in developing the SENACYT infrastructure grant proposal that led to the construction and operation of LIRT, as well as Neries De La Cruz, Geneva Montiel, and Tony Castillo for construction and equipment supply. The authors thank Giovanni Castillo and Maybis Garay for their contributions as grant fellows during LIRT's kickoff stage. The authors also thank Mayra Návalo, Aurelio Pineda, and Edgar Coto from Ministerio de Salud, and Ileana Rodríguez, Dámaso Muñoz, administrators at INDICASAT-AIP, for their valuable support in administering and operating LIRT. The authors thank Ing. Francisco García and Ing. Milagro Mainieri from SENACYT for their close evaluation and supervision in achieving the goals of this infrastructure grant. The authors also thank clinic and hospital personnel for their valuable assistance in providing access to laboratory registry books and supporting data collection. This study was also supported by the Sistema Nacional de Investigación (SNI) and the FIED grant No. 137-2023, including infrastructure funding under the Secretaría Nacional de Ciencia, Tecnología e Innovación SENACYT) project INF-11-39.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
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| AR | Auramine–rhodamine |
| CSS | Caja de Seguro Social |
| EQA | External quality assessment |
| HIV | Human immunodeficiency virus |
| INDICASAT-AIP | Instituto de Investigaciones Científicas y Servicios de Alta Tecnología-Asociación de Interés Público |
| IQR | Interquartile range |
| LCRSP-ICGES | Laboratorio Central de Referencia en Salud Pública, Instituto Conmemorativo Gorgas de Estudios de la Salud |
| LIRT | Laboratorio Interinstitucional Regional de Tuberculosis |
| MGIT | Mycobacteria Growth Indicator Tube |
| MINSA | Ministerio de Salud |
| NPV | Negative predictive value |
| PPV | Positive predictive value |
| TAT | Turnaround time |
| TB | Tuberculosis |
| WHO | World Health Organization |
| ZN | Ziehl–Neelsen |

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